Leading Edge
Minireview
Cell

When Size Does Not Matter: Pairing Sites during Meiosis

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DOI: 10.1016/j.cell.2005.11.031

Specialized sites along DNA maintain the pairing of homologous chromosomes during meiosis. Three research articles, including two in this issue of *Cell*, describe proteins from the fruit fly (Thomas et al., 2005) and the worm (MacQueen et al., 2005; Phillips et al., 2005) that bind to these sites and stabilize pairing interactions.

The alignment of homologous chromosomes that occurs early during prophase of meiosis usually occurs in two stages: a pairing stage where the homologs are roughly aligned and a synapsis stage where homologous chromosomes are aligned precisely and held together by a specialized structure, the synaptonemal complex (SC). Meiosis culminates in the segregation of homologous chromosomes that have been connected as a bivalent (a pair of duplicated homologous chromosomes). In most cases, this connection is generated by crossover events. The connection between homologs also dictates their segregation patterns, as each kinetochore of a bivalent is pulled in an opposite direction by the microtubules of the bipolar spindle (Hawley, 1988) (Figure 1).

Sites that are defined as having a role in meiotic recombination were originally called "pairing sites" and were used to explain the effects of translocations on crossing-over in the fruit fly *Drosophila* (Hawley, 1980). Instead of requiring large-scale or frequent comparisons of DNA sequences, homolog pairing is driven by a group of sequences at localized sites that are relatively short. In two organisms, *Drosophila melanogaster* and the worm *Caenorhabditis elegans*, the existence of specialized pairing sites is confirmed by three studies published in *Cell*, from McKee and colleagues (Thomas et al., 2005) and Dernburg and colleagues (this issue, MacQueen et al., 2005; Phillips et al., 2005). Despite differences between *C. elegans* hermaphrodites and *Drosophila* males with respect to meiotic chromosome segregation, localized sites play a central role in the pairing and segregation of chromosomes in both species. Also included in these studies are descriptions of proteins that accumulate at these sites.

It is important to consider the definitions of pairing and synapsis (Zickler and Kleckner, 1999). In most organisms, including *C. elegans*, synapsis is defined by the formation of the SC, which consists of proteins that attach homologous chromosomes in precise alignment along their entire lengths at a distance of separation of approximately 100 nm. In addition, there is also synapsis-independent pairing (sometimes referred to as presynaptic alignment or homolog juxtaposition), which refers to the long-distance recognition of homology often in the absence of obvious physical connections. The accuracy of this pairing is variable, may be required for synapsis, and can result in the alignment of homologs at a distance of 200 to 400 nm. *Drosophila* males are unusual because they do not have

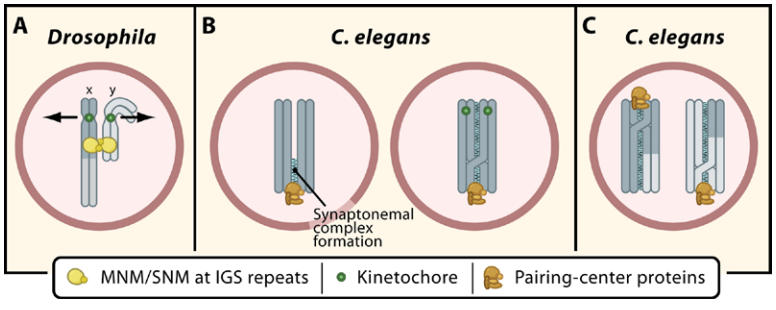

Figure 1. Contrasting Homolog Pairing in *Drosophila* Males and *C. elegans*
(A) The X and Y chromosomes of *Drosophila* males rely on a heterochromatic site (the intergenic spacer [IGS]) for homolog pairing and segregation. A single site may function both in pairing stabilization and as the attachment site for directing segregation (as shown by the arrows). This mechanisms works even when the kinetochores are not homologous, as with the X-Y pair.
(B) In *C. elegans*, the pairing center might have two functions. The first may be to stabilize presynaptic alignment. Although presynaptic alignment may occur via transient interactions along the length of each homolog, it is stabilized at the pairing center. The second function may be to promote SC formation. The SC is then required for crossing-over (right panel). Unlike *Drosophila* males, the pairing site does not also function as an attachment point to direct segregation, which is the function of the crossover.
(C) The two chromosomes in a reciprocal translocation synapse completely along their entire length. The single pairing center is also sufficient to drive synapsis through chromosomal regions that are either homologous (same color) or nonhomologous (different colors). Crossing-over is only observed in the synapsed regions that are homologous.

SCs and therefore only have SC-independent pairing between chromosomes.

Meiotic Pairing Sites in *Drosophila* Males

When the meiotic chromosomes of *Drosophila* males condense prior to metaphase, the X-Y bivalent (but not the autosomes) is joined by a single thread-like structure of heterochromatin known as the collochore (Cooper, 1964). Subsequent studies on X-Y pairing and segregation have focused on this single site. Work by McKee and coworkers found that a 240 bp intergenic spacer located between the ribosomal DNA (rDNA) repeats mediates disjunction of the X and Y chromosomes (McKee et al., 1992) (Figure 1A). The efficiency of segregation is dependent on the copy number of the intergenic spacer. Normally there are 200 to 250 copies of the rDNA transcription unit, but the ectopic insertion of as few as 5 to 8 copies of the intergenic spacer on the X chromosome can substantially improve segregation of an X-Y pair when the endogenous rDNA on the X is deleted. The autosomes, however, do not contain any rDNA, and their segregation likely depends on multiple discrete pairing sites (Cooper, 1964; McKee et al., 1993).

In their new study, Thomas et al. (2005) have found two genes—*stromalin in meiosis* (*snm*) and *modifier of mdg4 in meiosis* (*mnm*)—that appear to act at pairing sites in male flies. The SNM protein shares homology with SCC3, a factor essential for sister-chromatid cohesion, whereas MNM is a protein containing BTB domains produced from a locus that generates more than 30 different transcripts through alternative splicing. The C terminus of the isoform may confer the ability to bind to chromosomes during meiosis.

Mutants of the *snm* and *mnm* genes do not affect pairing during early meiotic prophase. Although there is no synapsis, early meiotic prophase is marked by an increase in the pairing of homologous chromosomes relative to premeiotic cells (Vazquez et al., 2002) Instead, the effect of mutations in *snm* and *mnm* is not seen until later in prophase, when homologs move into distinct territories and pairing is relaxed. Studies using fluorescence in situ hybridization (FISH) reveal that the homologous chromosomes in *snm* and *mnm* mutants are farther apart than in wild-type flies, demonstrating that *snm* and *mnm* normally prevent the homologs from separating prior to anaphase I. Thus, these data do not indicate whether SNM and MNM have a role in initiating homolog pairing, but they do show that these proteins have a role in the maintenance of homolog pairing. In this way, SNM and MNM help to substitute for the absence of crossovers during meiosis in *Drosophila* males. Further studies may reveal clues as to what initiates the physical connections between homolog pairs. The authors suggest that, prior to conjunction, the homologs are maintained in close proximity by transient interactions at SNM/MNM binding sites. This leads to the prediction that, for the X-Y pair, a FISH experiment using the intergenic spacer should show better pairing than a locus positioned in distal euchromatin.

Despite its homology to SCC3, SNM protein is probably not involved in cohesion but instead may be a substitute for chiasmata in homolog conjunction. Given the current understanding of the cohesin complex, it is tempting to imagine a cohesin-like mechanism for SNM and homolog conjunction. There do not, however, appear to be other divergent proteins in the cohesin complex in the *Drosophila* genome that interact with SNM. Instead, MNM is a possible interacting protein because SNM and MNM colocalize and are mutually dependent on each other for localization to chromosomes.

Both SNM and MNM localize to a single locus, the rDNA repeats on the X chromosome, during late prophase after the chromosomes have condensed. In contrast, the MNM protein is observed at multiple autosomal loci. Furthermore, the binding of MNM to the autosomes, but not to the X and Y chromosomes, depends on the *Teflon* gene. The *Teflon* gene is required for disjunction of the autosomes, but not the X-Y chromosomes (Tomkiel et al., 2001). Thus, these cytological results nicely mirror the genetic results, suggesting marked differences in the segregation of the X-Y chromosomes compared with segregation of autosomal pairs.

Meiotic Pairing Sites in *C. elegans*

One region near the end of each *C. elegans* chromosome promotes crossing-over (Herman and Kari, 1989; McKim et al., 1988). For example, fragments of the X chromosome containing sequences near one end engage in high levels of crossing-over, whereas chromosome fragments, no matter how large, that are detached from this region rarely undergo crossing-over. On the standard genetic map for *C. elegans*, this region is commonly referred to as the "left end" of the chromosome. The important question was whether these sequences, called pairing centers, were involved in presynaptic alignment, synapsis, or the loading of factors required for recombination. The two papers by the Villeneuve and Dernburg groups in this issue of *Cell* have gone a long way toward answering this question (MacQueen et al., 2005; Phillips et al., 2005).

A previous study had already shown that the pairing-center sequences at one end of the X chromosome had a role in the pairing process (MacQueen et al., 2002). In the absence of the SC component SYP-1, a transverse filament protein, the pairing center of the chromosome had the capacity to maintain pairing, whereas the rest of the chromosome paired no better than a mitotic cell. This showed that this region of the chromosome is capable of maintaining a paired state in the absence of SCs. MacQueen et al. (2005) have now shown that worms that lack most or all of the pairing center of the X chromosome, *meDf2* homozygotes, have defects in both the maintenance of homolog pairing in prophase and SC formation. However, homolog pairing in early prophase, when presynaptic alignment may be occurring, is close to normal. Therefore, the authors favor a model in which the pairing that occurs prior to synapsis (i.e., presynaptic alignment) is stabilized at the pairing centers.

A defect in presynaptic alignment could be the cause of the synapsis defects. However, several lines of evidence support a second function for the pairing center in promoting SC formation (Figure 1B). The X chromosomes do

occasionally synapse in worms that are either heterozygous or homozygous for *meDf2*. SC formation in *meDf2* heterozygous worms is fairly common, but these mutants appear to be unable to maintain presynaptic alignment in the absence of SCs. Therefore, this genotype may represent a situation where the presynaptic alignment and SC initiation functions have been separated. Due to the inability to stabilize presynaptic alignment, SC formation in *meDf2* heterozygous worms may be inefficient. However, when the SC does form, partially synapsed chromosomes are not observed. Therefore, it appears that, once synapsis is initiated, it almost always continues to the end of the chromosome. Similarly, a FISH experiment demonstrated that any sequence attached to a pairing center can form an SC. Thus, in a translocation heterozygote, sequences linked to a pairing center will form homologous SCs, but the sequences distal to a breakpoint will form nonhomologous SCs (Figure 1C). These results suggest that the pairing center is responsible for SC formation and that, once started, it continues without regard to homology. It is as if the pairing center initiates the correct "reading frame" for the SC, after which point homology is not checked. These results also are consistent with the genetic finding that the boundary for the suppression of crossovers in translocation heterozygotes corresponds precisely with the breakpoint, occurring only in the regions joined by homologous SCs (McKim et al., 1988).

In the second paper, Dernburg and colleagues (Phillips et al., 2005) show that the pairing centers are specific chromosomal loci that are bound by proteins such as HIM-8, which encodes a C2H2 zinc-finger protein. Mutants of *him-8* have severe reductions in crossing-over that are specific to the X chromosome. Furthermore, *him-8* mutants have a chromosome-specific pairing and synapsis defect. Thus, the phenotype of *him-8* mutants is very similar to *meDf2* homozygotes. It is exciting that HIM-8 protein localizes to the left end of the X chromosome, the site previously described as having pairing-center activity. Another exciting possibility for future work is to determine whether the family of *C. elegans* genes related to *him-8* could have similar functions in the pairing and synapsis of the autosomes.

him-8 mutants have stronger effects than *meDf2* mutants, which raises important questions at the core of the function of pairing centers. *him-8* mutants do not exhibit early SC-independent and transient pairing that is seen in *meDf2* mutants, suggesting that *him-8* mutants are defective in initiating as well as stabilizing presynaptic alignment. The authors hypothesize that presynaptic alignment depends on transient pairing interactions that occur along the length of the X chromosome and that HIM-8 is required for this process as well as for pairing-center activity. These transient pairing interactions would be stabilized at the pairing center. An alternative interpretation of their results is that the *meDf2* chromosome has only partially lost activity of the pairing center, whereas in *him-8* mutants, activity of the pairing center is lost completely. Future studies will determine whether homologous chromosomes initially interact at these sites or whether chromosomes first identify each other by a more general mechanism. The current studies mostly involve the X chromosome, which does have some unique features (e.g., McKim et al., 1993). It will be instructive when the autosomal sites receive similar scrutiny and when other types of rearrangements are studied. Does transient pairing initiate along the length of each homologous chromosome, or does it occur first at one end? Indeed, there is currently little evidence for presynaptic alignment along the length of the X chromosome in wild-type *C. elegans*. Answering these questions will likely require real-time analysis to determine whether *him-8* mutants have a stabilization defect rather than a defect in pairing.

The Relationship between Pairing, Synapsis, and Double-Strand-Break Formation

The nature of the sites that initiate SC formation is poorly understood in most organisms. Homolog synapsis can occur by double-strand-break (DSB) dependent or DSB-independent mechanisms, depending on the organism (Keeney, 2001). When DSBs are required, the mechanism by which they promote synapsis is not known, but DSBs could promote SC formation or stabilize homolog pairing, which, interestingly, are roles that are suggested for the pairing centers in *C. elegans*. Even less is known about DSB-independent SC formation.

It has been proposed in budding yeast that SC initiation occurs at the sites where crossovers will occur (Fung et al., 2004). Although in *C. elegans* this activity may only occur at the pairing centers, whether other organisms use specialized sites for SC initiation is not known. Specialized chromosomal sites that are required for meiotic crossovers have been mapped in *Drosophila* females, which have a conventional meiosis with DSBs and SC formation (Hawley, 1980; Sherizen et al., 2005). Interestingly, the current evidence does not rule out the possibility that the single worm site and the multiple sites on each chromosome in *Drosophila* females have similar functions. These sites could load SC components or other proteins such as those that may regulate crossover formation. Given that both *C. elegans* and *Drosophila* form SCs in the absence of DSBs, it is interesting to speculate that the use of specialized sites instead of crossover sites for stabilizing pairing and/or initiating SC formation defines the difference between those organisms that do not require DSBs for SC formation and those that do.

Is homolog pairing a prerequisite for DSB formation, thus ensuring that breaks occur only when a homolog is present, given that, in *C. elegans*, SCs form in the absence of DSBs? Probably not, because staining for Rad51 suggests that DSBs occur on the asynapsed X-chromosomes in both *meDf2* and *him-8* mutants. However, in these mutants, there is a defect in repairing the DSBs and an absence of crossovers. Ironically, the original descriptions of pairing centers were based on the suppression of crossovers. It was inferred from these studies that a reduction in crossovers indicated a defect in pairing. The truth is likely that the effects on crossovers are secondary. The failure to form SCs is the cause for the reductions in crossovers

because DSBs are apparently made, but they cannot be repaired in such a way as to yield crossovers. Another striking result is that even though DSBs are made in *meDf2* and *him-8* mutants, they are not sufficient to promote pairing or synapsis. Meiosis in *C. elegans* appears to be designed to prevent SC formation unless the sequences are linked to the pairing center.

Whereas *Drosophila* males use a single X-Y pairing site as a solution for two problems, the absence of SCs and chiasmata, why is there only a single pairing center on each chromosome in *C. elegans*? MacQueen et al. (2005) suggest that these single sites ensure that chromosome fragments are not efficiently segregated. Another possibility is that the existence of only a single site may be related to the high degree of crossover interference in *C. elegans* and the need for a discrete kinetochore. Although *C. elegans* mitotic chromosomes are holokinetic, there is a mechanism to restrict kinetochore activity to one end of each chromosome at meiosis I (Albertson and Thomson, 1993). Although either end of each *C. elegans* chromosome can possess the kinetochore activity, it is not understood how only one end is defined at each meiosis. At the same time, crossing-over is tightly regulated in *C. elegans*, typically one crossover per chromosome (Meneely et al., 2002). Perhaps a single pairing site is part of the mechanism to ensure that a single crossover occurs, or to define the single microtubule attachment site, or both.

The Question of Pairing or Stabilization

These three papers present the strongest evidence to date that there are specialized sites that are involved in homolog pairing and synapsis and that operate in a recombination-independent manner. Furthermore, these three studies propose functions for their respective pairing sites that are strikingly similar. Synapsis-independent stabilization of pairing by the pairing center in worms is similar to the proposed role of the MNM and SNM proteins in *Drosophila* males. In both cases, initial SC- and recombination-independent pairing interactions that occur on a chromosome-wide basis need to be stabilized. An important difference is that SNM and MNM bind many sites in the genome, suggesting there is a common function specified by each of the pairing sites in *Drosophila* males. In contrast, the HIM-8 protein binds to only one site, suggesting that the pairing center in *C. elegans* defines a feature of the chromosome that is unique in the genome.

Although meiosis in both *Drosophila* males and *C. elegans* has some unique features, these results are relevant to meiosis in most other systems. Rather than highlighting the forces that bring homologs together in the first place, these papers stress the importance of stabilizing the initial pairing interactions. Although there is an increase in homolog pairing early in meiotic prophase in the *Drosophila* male, whether this depends on the pairing sites is not known. Experiments similar to those by Vazquez et al. (2002), which monitored the pairing of chromosomal loci in real time, should reveal insights into this question. In *C. elegans*, it is proposed that a mechanism that does not involve the pairing center is responsible for the presynaptic alignment of homologs. In both organisms, the mechanisms for these early pairing interactions are clearly SC and recombination independent. Although some organisms depend on DSBs for SC formation and others do not, the important difference in how organisms begin meiotic prophase may not be in how their chromosomes pair but in how the initial pairing interactions are stabilized (either recombination dependent or independent). The identification of proteins that localize to the pairing sites in *Drosophila* males and *C. elegans* suggests there is a promising future in the quest to understand the mechanisms for recombination-independent pairing and SC formation. These mechanisms will be applicable to most other organisms, even those with DSB-dependent pairing and synapsis.

REFERENCES

Albertson, D.G., and Thomson, J.N. (1993). Chromosome Res. *1*, 15–26.

Cooper, K.W. (1964). Proc. Natl. Acad. Sci. USA *52*, 1248–1255.

Fung, J.C., Rockmill, B., Odell, M., and Roeder, G.S. (2004). Cell *116*, 795–802.

Hawley, R.S. (1980). Genetics *94*, 625–646.

Hawley, R.S. (1988). In Genetic Recombination, R. Kucherlapati, and G. Smith, eds. (Washington, DC: American Society of Microbiology), pp. 497–527.

Herman, R.K., and Kari, C.K. (1989). Genetics *121*, 723–737.

Keeney, S. (2001). Curr. Top. Dev. Biol. *52*, 1–53.

MacQueen, A.J., Colaiacovo, M.P., McDonald, K., and Villeneuve, A.M. (2002). Genes Dev. *16*, 2428–2442.

MacQueen, A.J., Phillips, C.M., Bhalla, N., Weiser, P., Villeneuve, A.M., and Dernburg, A.F. (2005). Cell, this issue.

McKee, B.D., Habera, L., and Vrana, J. (1992). Genetics *132*, 529–544.

McKee, B.D., Lumsden, S.E., and Das, S. (1993). Chromosoma *102*, 180–194.

McKim, K.S., Howell, A.M., and Rose, A.M. (1988). Genetics *120*, 987–1001.

McKim, K.S., Peters, K., and Rose, A.M. (1993). Genetics *134*, 749–768.

Meneely, P.M., Farago, A.F., and Kauffman, T.M. (2002). Genetics *162*, 1169–1177.

Phillips, C.M., Wong, C., Bhalla, N., Carlton, P.M., Weiser, P., Meneely, P.M., and Dernburg, A.F. (2005). Cell, this issue.

Sherizen, D., Jang, J.K., Bhagat, R., Kato, N., and McKim, K.S. (2005). Genetics *169*, 767–781.

Thomas, S.E., Soltani-Bejnood, M., Roth, P., Dorn, R., Logsdon, J.M., Jr., and McKee, B.D. (2005). Cell *123*, 555–568.

Tomkiel, J.E., Wakimoto, B.T., and Briscoe, A., Jr. (2001). Genetics *157*, 273–281.

Vazquez, J., Belmont, A.S., and Sedat, J.W. (2002). Curr. Biol. *12*, 1473–1483.

Zickler, D., and Kleckner, N. (1999). Annu. Rev. Genet. *33*, 603–754.